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Quarterly Status Report No. 2

Gentlemen:

This is a summary of research work performed during the period 19 June to 18 September 1963 on Contract NASw-648. Our work is proceeding according to schedule, and we expect to complete the contract requirements in the allotted time period.

(2)

Sincerely,

Aerospace Research Dept.

V. W. Greene 8 Oct. 1963
Project Scientist

T
RESEARCH TO DETERMINE THE EXISTENCE AND IDENTITY OF VIABLE MICROORGANISMS IN THE STRATOSPHERE

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SUMMARY

During this quarter, the following tasks were accomplished:

1) Final evaluation of the first flight (11 May 1963)

2) Preparation, launch, recovery, and analysis of the second flight (31 July 1963)

3) Evaluation of reliability of data from all flights carried out under this program and NASr-81

4) Simulation experiments to aid interpretation of stratospheric data

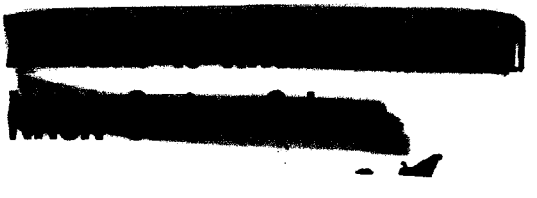
5) Initiation of preparations for the third flight.

We are presently preparing for another balloon launch in October and are concurrently performing experiments on the reliability of our equipment and program concepts.

EVALUATION OF MAY 11th FLIGHT

In our previous report we indicated the possibility of sealing-mechanism malfunction, and the consequent masking of the stratospheric sample by ground-borne dust during impact. A detailed examination of the organisms recovered from the samplers and their comparison with environmental samples taken from the ground at the impact site strongly support this possibility. A recheck of the closing apparatus in the altitude chamber indicated that we put too much spring tension on the trip-lock assembly and that the horizontal tension on the shaft prevented a smooth vertical closure of the sealing pans. A gap of several millimeters remained between the pans and sampler throat and permitted access of aerosolized dust during impact. The sterile control, which was closed and locked before ascent, showed only negligible contamination.

Of some significance to the interpretation of previous data, and perhaps to other experiments of this nature, is the quantitative difference between



the contamination levels on each filter and on the individual filter segments. Although all three samplers were contaminated with extraneous dust, there was more than an order of magnitude difference between them, signifying either a random statistical variation or (more probably) the difference in impact sequence. Because the four samplers are suspended on the corners of the gondola and the payload impacts while moving both vertically and horizontally, each of the sampling units is subjected to different stress loads. The sterile control is not necessarily comparable, therefore, with its sampling counterparts, nor is contamination data from one active sampler necessarily comparable with that from another.

PREPARATION FOR FLIGHT NO. 2

The payload from flight no. 1 had been recovered in suitable condition and was prepared for another flight. The mechanical self-closing apparatus was overhauled, suitable springs were employed to overcome the malfunctions of the part, the blowers and flowmeters were recalibrated, and the electronic programming equipment was renovated.

Two important modifications of the experiment were made:

1) Polyurethane Anti-Contamination Seals - In the past flights, we employed metal sealing pans gasketed with either a thin strip of polyurethane foam or silicone rubber. Although these pans worked well in the laboratory, we were not entirely satisfied with their reliability under stratospheric conditions. For this flight we consequently replaced the pans with "plugs" made from overlapping layers of polyurethane foam. These plugs could be autoclaved and seated themselves fairly well against a silicone rubber gasket in the sampler throat although they became stiff and unpliable at -50 C. Repeated experiments in the altitude chamber indicated that the units could close and lock themselves quite adequately with these closures and that they could effectively minimize external contamination.

2) Internal Diffusion Controls - In an attempt to discriminate between organisms sampled in the stratosphere and organisms accidentally entrained after impact, we taped small pieces of

polyurethane foam to the inside walls of the samplers, both upstream and downstream from the filter pad. We thought that the large filter pad would show both stratospheric organisms (trapped from the high velocity airstream) and any leaking past the seals (by diffusion), whereas the taped foam would show only the latter (since these segments of foam were not in the streamline).

We subsequently recognized that the turbulence created in the airstream when it passed the sealing pan and the throat of the sampler could cause some impaction of stratospheric particles on the internal skin (and the internal controls). We are now performing simulation tests in the environmental chamber to ascertain the effectiveness of these internal controls and to interpret the counts we found on them.

FLIGHT NO. 2 (NASA 4)

On 31 July, a probe was launched from New Brighton, Minnesota. The samplers rose to 89,000 ft. The dust covers were jettisoned, and the balloon started its descent. Samplers 1 and 2 started at 85,000 ft and sampled for a few minutes at this altitude. Sampler 1 was shut off as scheduled by a timer switch; sampler 2 was shut off prematurely by a malfunctioning switch at about 72,000 ft and completed its collection at 40,000 ft. Sampler 4, which was hand-closed just prior to launch, served as a flight control. The payload impacted without damage in a wooded hillside near Spring Valley in Pierce County, Wisconsin. Our inspection at the impact site showed that all units were locked and sealed.

RESULTS OF FLIGHT 2 (NASA 4)

Sampler 1, which collected approximately 3,000 cu ft of ambient air at 85,000 ft yielded 13 bacterial and 22 mold colonies on the filter pad. Sampler 2, which collected approximately 5,000 cu ft at about the same altitude, yielded 18 bacterial and 52 mold colonies on the filter pad. Sampler 3,

which collected about 140,000 cu ft of air between 72,000 ft and 40,000 ft, yielded 29 bacterial and 71 mold colonies. The sterile control (#4) yielded 2 bacterial and 2 mold colonies.

The internal control pads from sampler 1 had 24 bacteria and 3 molds; that from sampler 2 had 7 bacteria and 5 molds; the pads from sampler 3 had 27 bacteria and 6 molds; and from the sterile control the pads had 30 bacteria and no molds.

The predominant organisms on the filter pads from all three sampling units (1, 2, and 3) were Cladosporium and Alternaria. The predominant organisms on the internal controls were spore-forming rods.

INTERPRETATION OF DATA

At this stage of the project, it might be worthwhile to evaluate the results of all four flights (two on Contract NASw-648 and two on Contract NASr-81).

In no case have we carried out an experiment that was an unqualified success. We are not yet able to make an unequivocal statement about the existence and identity of microorganisms in the stratosphere. Our biggest problem thus far was anticipated but has not yet been completely solved--post impact contamination, and its possible masking of stratospheric organisms. We think that we have eliminated from serious consideration most other sources of contamination. But until we can definitely establish that perfect sealing has taken place before impact and that the integrity of the unit remains unimpaired during the stress of impact, the interpretation of data must remain speculative.

We believe that the last flight was the best of the series. Even though some contamination may have occurred, the very low levels encountered permit us to make some reasonable estimates about the quantity of organisms in the stratosphere. The sampler which collected over 100,000 cu ft

of air had about 100 organisms on its filter pad. If every one of these organisms was trapped in the stratosphere, the count would be approximately 1/1000 cu ft (ambient). If every one of these organisms was extraneous contamination, the stratospheric count would be $<$1/1000 cu ft (ambient). This general value was confirmed during the second flight on Contract NASr-81, during which 108 colonies were recovered from a sampler exposed to 140,000 cu ft.

The real discrepancy in all of our data thus far is the count obtained from the high altitude sampler on the first flight in August 1962. From that unit we recovered about 2×10^4 organisms. On the basis of our subsequent experience, we feel that the sampler may have been contaminated, although it will be difficult to confirm or disprove this belief.

In general, we find that every flight provides us with further valuable experience and an opportunity to evaluate the previous experiments more reasonably. We do not yet believe that stratospheric bioaerosol sampling is a routine technique for data collecting and interpretation. We do not think it wise to draw conclusions from any given probe until the entire program has been completed and thoroughly evaluated.

OTHER WORK

We are now actively engaged in a series of simulation experiments to gain further knowledge about the behavior of our samplers and the validity of our assumptions and techniques. We are measuring the contamination that can be entrained in the samplers during the jettisoning of our dust covers and shrouds. We are critically evaluating the sterilization procedures employed for the samplers, both the autoclaving and ethylene oxide treatment. We are checking a variety of closing and sealing systems that could be employed. And we are studying the survival of lyophilized organisms under exposure to low-pressure, high-velocity airstreams.

FUTURE WORK

We are preparing the equipment for the third flight in this series (NASA 5) and hope to be ready for launch before the end of October.